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Response of isolated Dahl rat kidney to calcium antagonists

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Response of isolated Dahl rat kidney to calcium antagonists. We studied the responses of isolated perfused kidneys from prehypertensive, salt–sensitive (DS) and salt–resistant (DR) Dahl rats to nitrendipine or verapamil, after norepinephrine vasoconstriction. The perfusion pressure was kept constant. Superimposition of these calcium antagonists upon norepinephrine increased DS GFR by 155% and DR GFR by 58% ($P = 0.03$), with verapamil increasing the GFR more than nitrendipine ($P = 0.02$). Nitrendipine and verapamil also partially reversed norepinephrine induced increases in renal vascular resistance, but did not decrease vascular resistance or increase GFR in the absence of norepinephrine. During the increase in GFR produced by calcium antagonists, DR sodium excretion increased, but DS sodium excretion did not. Therefore, calcium antagonists disproportionately increased DS kidney GFR but did not correct DS kidney sodium retention. These data raise the possibility that the DS rat kidney possesses an abnormality of cell calcium regulation affecting glomerular dynamics, and provide evidence that the renal perfusion pressure is more critical than the GFR in adjusting DS rat sodium–excretion.

Humans with essential hypertension and the Kyoto spontaneously hypertensive rat (SHR) manifest abnormalities of calcium metabolism [1, 2]. Calcium metabolism in the Dahl salt–sensitive (DS) and resistant (DR) rat has been characterized less extensively. A great deal of work has focused on the tendency toward sodium retention by the DS rat kidney, a characteristic thought to be important in the pathogenesis of Dahl rat hypertension [3]. We have reported that isolated perfused kidneys from the SHR manifest exaggerated glomerular circulatory responses to the calcium channel blocker, verapamil [4]. Verapamil vasodilation largely reversed norepinephrine induced increases in SHR renal vascular resistance (RVR), and also increased the glomerular filtration rate (GFR) to values greatly exceeding those before norepinephrine. Wistar–Kyoto rat (WKY) kidney GFR values returned only to baseline levels [4].

In order to determine whether or not the phenomenon is unique to the SHR and is dependent upon the presence of hypertension, we have extended our observations to isolated kidneys from prehypertensive DS rats and their salt–resistant DR counterparts. Kidneys from prehypertensive DS rats manifested modestly–exaggerated glomerular responses to the calcium channel blockers, verapamil and nitrendipine, compared to DR kidneys. In contrast to the SHR kidney which manifested increased sodium excretion in proportion to the GFR after verapamil [4], DS kidney sodium excretion did not increase in response to an increased GFR.

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Methods

We isolated kidneys from 24 male DS and 22 male DR rats (obtained from Dr. J. Iwai, Brookhaven National Laboratory, Upton, Long Island, New York, USA) which had been maintained on a 0.3% NaCl chow diet. At the time of study, the age of the DS rats averaged 15 ± 2 weeks (mean ± SEM) and that of the DR rats averaged 14 ± 2 weeks. DS rat weight averaged 412 ± 11 g and DR rat weight averaged 411 ± 10 g. During Inactin anesthesia (100 mg/kg i.p.), the carotid or femoral mean arterial pressure was measured directly utilizing a pressure transducer system (Harvard Instruments, Millis, Massachusetts, USA). Our kidney isolation and perfusion procedures have been described previously [5]. Briefly, after kidneys were flushed and isolated without ischemia, they were perfused at 37°C with an oxygenated cell–free, modified Krebs Henseleit perfusate. Oxygenation was accomplished by equilibration with 95% O_2/5% CO_2; this also stabilized the perfusate pH at 7.40 to 7.42. The perfusate contained electrolytes, glucose, inulin, and amino acids in amounts reported previously [5]. Bovine serum albumin fraction V, (Pentex–Miles, Kankakee, Illinois, USA) had been purified previously with a hemodialyzer.

The hydraulic pressure of the perfusion system was monitored continuously and the renal perfusion pressure was maintained at 105 mm Hg by adjusting the perfusate flow rate. The renal perfusion pressure was determined by taking into account and subtracting off the pressure decrease imposed by the precalibrated needle cannulating the renal artery. After an initial 20 to 30 minutes of perfusion, urine and perfusate specimens were obtained for two control periods of 5 to 10 minutes each.

Sufficient norepinephrine then was added to the perfusate to increase the RVR by 50% over the control value. Simultaneously, an infusion of norepinephrine dissolved in perfusate was begun and adjusted to a rate sufficient to maintain the increased RVR for the duration of the experiment. After stabilization, urine and perfusate specimens were obtained for two more periods of five minutes each during norepinephrine infusion. Subsequently, either verapamil (0.01 mM) or nitrendipine (0.01 mM) was added to the perfusate, and similar specimens for a final experimental phase were obtained. In separate time control experiments, DS and DR kidneys were perfused similarly except that norepinephrine was omitted from the

perfusate vehicle; nitrendipine or verapamil was added after the same time interval had elapsed.

Norepinephrine (Parke–Davis, Morris Plains, New Jersey) and verapamil were dissolved directly in perfusate. Nitrendipine was dissolved in a small amount of polyethylene glycol insufficient to affect kidney function in preliminary experiments, and was protected from light exposure. Urine volumes were estimated gravimetrically. Inulin was analyzed chemically by a resorcinol method; its clearance provided an estimate of the GFR. Sodium was determined by flame photometry. RVR was computed as the quotient of the renal perfusion pressure divided by perfusate flow. Data were not factored by kidney weights; DS kidney weight averaged 1.83 ± 0.05 g compared to DR kidney weight of 1.70 ± 0.05 g ($P = 0.31$).

Statistical comparisons of experimental with control values were done by the paired t-test. The significance of differences among different experimental groups was determined by an analysis of variance. When significant overall differences existed ($P < 0.05$), all possible comparisons were made utilizing the Newman–Keuls multiple comparison procedure at a significance level of $P < 0.05$. When fewer comparisons were made, we used the Bonferroni procedure. Additionally, a multivariate analysis of variance with repeated measures was utilized in order to compare the influence of Dahl status (DS vs. DR), choice of calcium antagonist (verapamil vs. nitrendipine) and presence of agonist (norepinephrine vs. vehicle) on sequential and nonsequential changes in GFR, RVR, and sodium excretion. These analyses were performed using Systat^R programs (Systat, Inc., Evanston, Illinois, USA) on an IBM AT microcomputer. Data are expressed as mean ± SEM throughout.

Results

During anesthesia, mean arterial pressure of DS rats averaged 129 ± 4 mm Hg and that of DR rats averaged 114 ± 3 mm Hg ($P = 0.007$). The results from experiments with DS and DR kidneys receiving norepinephrine followed by either nitrendipine or verapamil are summarized in Figure 1 and in Table 1. DS kidney RVR was more sensitive to norepinephrine than DR kidney RVR ($P = 0.006$). In order to increase RVR by 50%, DS kidneys required a norepinephrine bolus of 57 ± 4 ng/ml followed by the constant infusion of 120 ± 10 ng/minute into 65 ml of recirculating perfusate. DR kidneys required 77 ± 6 ng/ml followed by 157 ± 19 ng/minute. The decreases in GFR of DS and DR kidneys were not affected differentially by norepinephrine ($P = 0.50$).

Calcium channel blockers then were added while norepinephrine infusion was continued. Verapamil both decreased the RVR and increased the GFR to a greater extent than nitrendipine ($P = 0.026$ and 0.004, respectively) when compared to values during norepinephrine infusion alone. Compared to the initial control phase, both verapamil and nitrendipine increased the GFR of DS and DR kidneys (Table 1). Only when both groups were pooled was the increase in GFR significantly greater in DS than in DR kidneys (Table 2). By analysis of variance of the effects of Dahl status and the particular calcium antagonist utilized, the GFR increased significantly more in DS than DR kidneys ($P = 0.033$) and verapamil increased the GFR more than nitrendipine ($P = 0.018$). The increments in GFR for the DS kidneys were approximately double those of the DR kidneys, and increments in GFR for verapamil treated kidneys

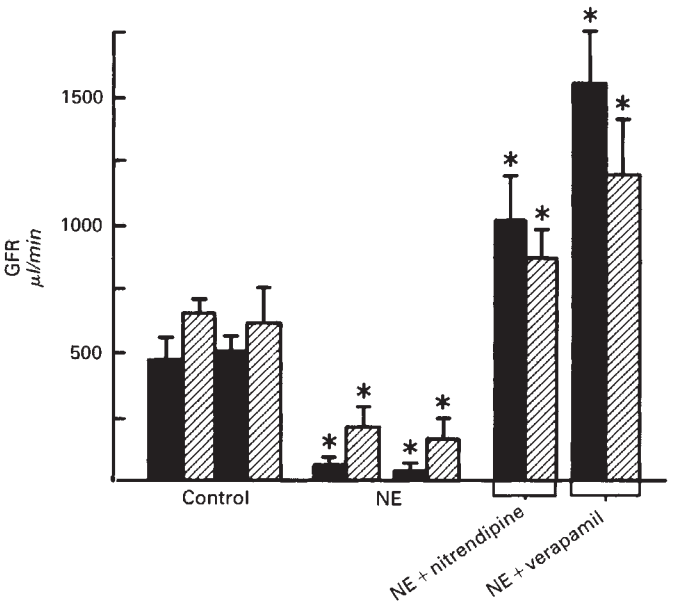

Fig. 1. *Glomerular response to calcium antagonist addition to perfusate of isolated norepinephrine (NE) vasoconstricted DS (■) and DR (▨) kidneys.* GFR increased more in DS than DR kidneys and verapamil increased GFR more than nitrendipine. Asterisks indicate significant differences from control (CON) phase ($P < 0.05$). These calcium antagonists did not increase GFR in the absence of norepinephrine. Nitrendipine and verapamil affected renal vascular resistance similarly.

were approximately double those of corresponding nitrendipine–treated kidneys (Table 2). Despite these differential influences on changes in GFR imposed by Dahl status and by the particular calcium antagonist, RVR and renal perfusate flow (RPF) changes (Table 2) were unaffected by Dahl status ($P = 0.37$) and choice of calcium antagonist ($P = 0.12$).

Although the calcium channel blockers collectively increased DS kidney GFR in an exaggerated fashion, parallel changes in DS kidney absolute and fractional sodium excretion did not occur (Table 2). Baseline values for sodium excretion did not differ significantly, but diverged in succeeding phases with DR tending to exceed DS, especially during the calcium antagonist phase (Table 1). Absolute sodium excretion increased in both groups of DR kidneys, but not in DS kidneys. Likewise, DS fractional sodium excretion (FE_{Na}) was less than DR FE_{Na} during the calcium antagonist phase ($P = 0.001$). Although nitrendipine accentuated differences between DS and DR sodium excretion and FE_{Na} more than verapamil, the two agents did not affect changes in these parameters from control differentially ($P = 0.9$ and 0.7, respectively). Comparisons of changes between the control and calcium antagonist phases indicated that there were significantly greater increases in both sodium excretion and FE_{Na} for DR than DS kidneys (Table 2). DS kidneys did not increase either parameter in spite of their large GFR increases.

We also examined the effects of verapamil and nitrendipine without norepinephrine in time control experiments (Table 3). Those studies encompassed the same time intervals as the experiments with norepinephrine. Neither calcium antagonist alone produced a significant increase in GFR or absolute sodium excretion in the absence of nonrepinephrine. Analysis

Table 1. Calcium antagonist superimposition upon norepinephrine

GFR *μl/min*			Na Excretion *μEq/min*			FE_{Na} × 100%			RVR *mm Hg/ml · min*$^{-1}$		
CON	NE	NE+CA	CON	NE	NE+CA	CON	NE	NE+CA	CON	NE	NE+CA
Nitrendipine											
DS kidneys (N = 8)											
466	58[a]	992[a]	2.43	0.42[a]	1.90	3.79	8.36	1.73[a]	2.21	3.39[a]	2.57[a]
± 94	± 27	± 135	± 0.58	± 0.15	± 0.40	± 0.61	± 3.46	± 0.39	± 0.10	± 0.15	± 0.14
DR kidneys (N = 7)											
647	217[a]	864[a]	3.75	1.69[a]	5.66[a]	4.36	4.71	4.98	1.94	3.09[a]	2.33[a]
± 52	± 53	± 90	± 0.75	± 0.57	± 1.01	± 0.74	± 0.94	± 0.88	± 0.08	± 0.17	± 0.13
P 0.128	0.015	0.460	0.181	0.040	0.003	0.560	0.359	0.004	0.051	0.407	0.240
Verapamil											
DS kidneys (N = 6)											
504	41[a]	1545[a]	1.57	0.24[a]	1.69	2.78	5.21	1.03[a]	2.31	3.48[a]	2.53[a]
± 83	± 9	± 157	± 0.56	± 0.07	± 0.26	± 0.47	± 1.88	± 0.23	± 0.19	± 0.31	± 0.20
DR kidneys (N = 5)											
612	172[a]	1187[a]	2.35	0.64[a]	3.58[a]	3.30	4.95	2.65	2.27	3.71[a]	2.58[a]
± 118	± 88	± 172	± 0.63	± 0.12	± 1.08	± 0.84	± 1.66	± 0.96	± 0.11	± 0.26	± 0.11
P 0.462	0.136	0.160	0.378	0.013	0.097	0.269	0.921	0.106	0.884	0.439	0.825
Overall DS vs. DR Comparisons (both drugs)											
P 0.086	0.003	0.168	0.108	0.015	0.001	0.267	0.373	0.001	0.176	0.916	0.447

Abbreviations are: GFR, glomerular filtration rate; FE_{Na}, fractional sodium excretion; RVR, renal vascular resistance; CON, control; NE, norepinephrine infusion; NE + CA, calcium antagonist superimposed on norepinephrine.
[a] Significant difference from control ($P < 0.05$). P values are DS vs. DR comparisons. Values are means ± SEM.

Table 2. Changes in renal hemodynamics and sodium excretion during calcium antagonist superimposition upon norepinephrine

Δ GFR *μl/min*	Δ Na Excretion *μEq/min*	Δ Fe_{Na} %	Δ RPF *ml/min*
Nitrendipine			
DS kidneys (N = 8)			
+526	−0.53	−2.06	−6.2
± 154	± 0.33	± 0.27	± 0.9
DR kidneys (N = 7)			
+217	+1.90	+0.62	−8.3
± 104	± 0.42	± 0.27	± 1.8
P 0.065	<0.001	<0.001	0.148
Verapamil			
DS kidneys (N = 6)			
+1040	+0.13	−1.22	−3.8
± 210	± 0.42	± 0.29	± 0.9
DR kidneys (N = 5)			
+575	+1.24	−0.65	−5.4
± 215	± 0.57	± 1.25	± 1.3
P 0.079	0.072	0.319	0.164
Overall DS vs. DR comparisons (both drugs)			
P 0.026	<0.001	0.002	0.085

Each Δ value is value during final phase (calcium antagonist plus neorepinephrine) minus control. Data are means ± SEM. P values are DS vs. DR comparisons.

of variance of all data (experimental and time control data combined) utilizing the presence or absence of norepinephrine as a variate indicated that the presence of norepinephrine was required in order to elicit an increased GFR ($P < 0.001$) and sodium excretion ($P = 0.002$), along with a residual increase in RVR ($P < 0.001$), after the addition of calcium antagonists. Examination of the changes between the control and calcium antagonist phases for all of the parameters of Table 3 indicated that there were no significant intergroup differences, with P ranging from 0.06 to 0.42. Additional time control experiments utilizing norepinephrine alone, without calcium antagonists, indicated a stable degree of norepinephrine induced vasoconstriction over the duration of these studies (Table 4).

Discussion

When verapamil or nitrendipine was superimposed upon norepinephrine infusion, the GFR of DS kidneys increased in a mildly exaggerated fashion compared to that of DR kidneys. Therefore, the results correspond to those from our previous experiments employing kidneys from normal Sprague–Dawley rats [5]. Those kidneys exhibited variable GFR responsiveness when verapamil or diltiazem was superimposed upon norepinephrine.

Although RVR was not affected differentially by verapamil and nitrendipine at equal concentrations, the GFR increased to a greater extent after the superimposition of verapamil than after superimposition of nitrendipine. This may have reflected different pharmacological properties of these agents. Nitrendipine and other dihydropyridine derivatives are thought to block transmembrane calcium fluxes rather specifically [6], but verapamil may exert additional influences on cell metabolism [7]. Dihydropyridine derivatives such as nitrendipine appear to bind specifically to slowly-inactivating vascular calcium channels while in the inactivated state [8]. The fact that both agents, taken together, manifested a qualitatively similar differential effect on DS kidney GFR is consistent with the possibility that an abnormality of cell calcium regulation exists in the DS rat kidney.

Despite greater increases in DS kidney GFR following calcium antagonist superimposition, DS sodium excretion remained unchanged and FE_{Na} decreased. Several previous studies have indicated that isolated DS kidneys exhibit "memory" with respect to sodium reabsorption, in that they manifest a blunted natriuresis over a range of perfusion pressures compared to DR kidneys [9–11]. In the present work in which a constant perfusion pressure of 105 mm Hg was maintained, the superimposition of verapamil or nitrendipine upon norepineph-

Table 3. Time control experiments without norepinephrine

GFR μl/min			Na excretion μEq/min			FE_{Na} × 100 %			RVR mm Hg/ml · min^{-1}		
CON-1	CON-2	CA	CON-1	CON-2	CA	CON-1	CON-2	CA	CON-1	CON-2	CA
Nitrendipine											
DS kidneys (N = 5)											
622 ± 83	529 ± 119	725 ± 96	2.55 ± 0.44	2.56 ± 0.50	2.66 ± 0.53	3.42 ± 0.84	3.89 ± 0.68	2.82 ± 0.32	1.97 ± 0.16	1.97 ± 0.18	1.99 ± 0.19
DR kidneys (N = 5)											
798 ± 93	641 ± 114	819 ± 33	6.59 ± 1.37	6.32 ± 1.39	6.24 ± 1.34	6.16 ± 0.99	7.02 ± 1.22	5.89 ± 1.33	1.70 ± 0.12	1.64 ± 0.13	1.65 ± 0.12
P 0.131	0.476	0.803	0.012	0.027	0.025	0.042	0.030	0.028	0.239	0.198	0.215
Verapamil											
DS kidneys (N = 5)											
593 ± 95	578[a] ± 77	563[a] ± 102	3.31 ± 0.99	2.99 ± 0.85	3.00 ± 0.86	3.46 ± 0.80	3.62 ± 0.79	3.77 ± 0.85	1.87 ± 0.05	1.82 ± 0.05	1.80 ± 0.05
DR kidneys (N = 5)											
551 ± 70	430[a] ± 50	376[a] ± 40	4.12 ± 0.70	3.93 ± 0.68	3.78 ± 0.57	5.85 ± 0.99	6.94 ± 1.14	7.60[a] ± 1.16	1.67 ± 0.08	1.61 ± 0.09	1.59 ± 0.09
P 0.260	0.010	0.126	0.523	0.663	0.470	0.099	0.043	0.028	0.073	0.070	0.083

Abbreviations are: CON-1 corresponds to control phase; CON-2 corresponds to norepinephrine phase; CA corresponds to final phase.
[a] $P < 0.05$ compared to CON-1. Values are means ± SEM. P values are DS vs. DR comparisons

Table 4. Norepinephrine time controls without calcium antagonists

	GFR μl/min			RVR mm Hg/ml · min^{-1}		
	CON	NE-1	NE-2	CON	NE-1	NE-2
DS kidneys (N = 3)	567[a] ± 85	36 ± 13	37 ± 15	2.43[a] ± 0.03	3.54 ± 0.06	3.38 ± 0.15
DR kidneys (N = 3)	675[a] ± 86	404 ± 110	414 ± 111	1.40[a] ± 0.03	2.13 ± 0.05	2.11 ± 0.07

NE-1 and NE-2 are sequential phases employing norepinephrine infusion without calcium antagonists.
[a] Significant difference from NE-1 phase ($P < 0.05$)

rine accentuated the differences between DS and DR natriuretic capacities (Tables 1, 2). The enhancing effect of these calcium antagonists on the GFR of DS kidneys was not accompanied by parallel changes in their sodium excretion. Although absolute sodium reabsorption increased in all four groups of kidneys as the GFR increased, the increase was greater for DS then for DR kidneys ($P < 0.001$). These results indicate that calcium antagonists failed to correct sluggish, DS kidney sodium–excretion.

Verapamil or nitrendipine superimposition upon norepinephrine did not return the RVR to control values. If residual increases in RVR of approximately 15% reflected increased mesangial contractility, a parallel increase in GFR would seem improbable. The residual increase in RVR instead may have reflected a persistent component of norepinephrine–induced efferent arteriolar–vasoconstriction during calcium channel–blocker superimposition [12]. However, our results do not allow an assessment of the mechanisms underlying changes in glomerular dynamics.

Several investigators have shown that the DS rat kidney achieves a very blunted natriuresis in response to an increasing perfusion pressure [9–11]. Our results indicate that large increases in the GFR do not increase DS kidney sodium excretion in the absence of an increase in perfusion pressure. In contrast, DR kidney sodium excretion increased briskly under similar circumstances. The modest increases in DS kidney sodium excretion accompanying stepwise elevation of the renal perfusion pressure during other isolated perfused Dahl kidney experiments probably can be attributed to hydraulic pressure increases alone, and not to pressure induced increases in the GFR per se. To the extent that the isolated kidney preparation reflects events in the living animal, the renal perfusion pressure appears to be fundamentally involved in achieving DS rat sodium balance. A report employing lithium as a marker for proximal tubule sodium–reabsorption has suggested that the latter is accentuated in the DS rat kidney [13]. If so, it would be interesting to determine whether DS proximal tubule reabsorption is less sensitive to hydraulic pressure changes than is proximal reabsorption in DR rats.

Sodium chloride–induced hypercalciuria has been reported in DS rats [14] and in DOCA-salt hypertensive rats [15]. More recently, Umemura et al have reported a depressed renal adenylate cyclase response to parathyroid hormone (PTH) in the Rapp variant of the DS rat and have postulated that this defective response to PTH may be the cause of DS rat hypercalciuria [16]. Although unproven, one can speculate that calcium depletion could play a role in the pathogenesis of hypertension [1]. In addition, because most currently available data suggest that renal sodium transport varies inversely with the cytosol calcium [17–19], it is possible that calcium depletion

may contribute to avid sodium reabsorption by the DS rat kidney.

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